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EXTERNAL PEER REVIEW OF EPA'S DRAFT AQUATIC LIFE SCREENING VALUES FOR N-(1,3-DIMETHYLBUTYL)-N′-PHENYL-P-PHENYLENEDIAMINE (6PPD)

FINAL PEER REVIEW SUMMARY REPORT

November 2023

Submitted to:
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CONTENTS

1.0 INTRODUCTION

This report documents the results of an independent external peer review of the U.S. Environmental Protection Agency's (EPA) draft *Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater*.

ERG (a contractor to EPA) organized this review and developed this report. The report provides background on the development of the draft document (Section 1.1), describes ERG's peer reviewer selection process (Section 1.2), provides reviewers' comments organized by charge question (Section 2.0) and additional comments (Section 3.0). Appendix A provides the charge to reviewers and Appendix B presents the reviewer comments organized by reviewer.

1.1 Development of the Draft Documents

The purpose of EPA's preliminary *Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater* is to provide EPA's scientific rationale for the draft screening values for 6PPD focused on the protection of aquatic life. The criteria are designed to solely protect sensitive aquatic life and is based on the best available data and best professional scientific judgements on the toxicological effects of 6PPD to aquatic life.

N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine (6PPD) is an additive to vehicle tire rubber, where it functions to protect rubber from reactions with ozone and oxygen, which can lead to degradation and cracking. Recent research has determined the ozonation of (6PPD) produces the reaction product 6PPD-quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) through hydrolysis and photodegradation. 6PPD-quinone recently has been shown to present an environmental threat based on its toxicity to salmon, where it has been identified as the causal agent in urban runoff mortality syndrome (URMS) observed in the Puget Sound area of Washington state.

Relatively limited 6PPD and 6PPD-quinone toxicity studies have been conducted on various aquatic taxa, including numerous species of fish and invertebrates, indicating that exposure to 6PPD and 6PPD-quinone through the aquatic environment causes population level effects, particularly mortality to acute exposures across aquatic life in the case for 6PPD and to fish such as salmonids, which appear to be sensitive to 6PPD-quinone exposures. In these drafts, EPA provides support for and outlines the derivation of screening values for 6PPD and 6PPD-quinone that would be protective of sensitive aquatic life.

This report is a compilation of external peer review comments received for 6PPD. A separate external peer review of the *Aquatic Life Screening Values for N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone (6PPD-quinone) in Freshwater* was conducted and the peer review comments received for 6PPD-quinone are compiled in a separate report.

1.2 Peer Reviewers

For this review, ERG identified, screened, and selected reviewers who had no conflict of interest in performing the review and who collectively met the following technical selection criteria provided by EPA:

ERG initiated a search process, asking interested candidates to describe their qualifications and respond to a series of "Conflict of Interest" (COI) analysis questions. ERG carefully screened submissions to identify a pool of qualified, COI-free candidates. From the set of candidates who met the criteria, ERG proposed a pool of five candidates to EPA on October 17, 2023. From this pool, ERG selected three experts who collectively best met the selection criteria. ERG contracted with and committed the following three experts to perform the review:

- **Jerry Diamond, Ph.D.**; Director of Ecotoxicology, TetraTech
- **David M. Janz, Ph.D.**; Professor, University of Saskatchewan
- **Richard Grippo, Ph.D**.; Emeritus Professor of Environmental Biology, Arkansas State University

ERG provided reviewers with instructions, the draft *Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater*, and the charge to reviewers (Appendix A of this report) prepared by EPA. Reviewers worked individually to develop written comments in response to the charge questions. After receiving reviewer comments, ERG compiled responses by charge question (see Section 2.0) and included the responses organized by reviewer (see Appendix B).

2.0 REVIEWER COMMENTS ORGANIZED BY CHARGE QUESTION

This section organizes reviewer comments by charge question (see Appendix B for reviewer comments organized by reviewer).

2.1 Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD to aquatic life.

2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life	
Reviewer	**Comments**
Reviewer 1	The document is generally well written and flows logically. The background summary of environmental fate and distribution of 6PPD was good. The explanation of how EPA derives water quality criteria was clear. I have provided a marked-up version with minor editorial suggestions and comments. There are several inconsistencies in the descriptions of experimental procedures when summarizing the various studies. The document must be consistent and specific when describing the control treatments among studies. In all instances, specify "solvent control" or "solvent vehicle control" when such a control was employed, and when known state the solvent concentration occurring in the actual exposure and control vessels as a percentage volume/volume. For example, I was confused on page 44 lines 5-7 ("No mortality was observed in the control or treatment concentration. Mortality in the control was 5%, and mortality in the test concentration was 100%"). I am assuming that "treatment concentration" refers to the solvent control? In addition, please specify what is meant by a negative control. Is this water-only, without solvent added? In the study descriptions (initial sentence of each) be consistent and always state whether 6PPD was measured or unmeasured.
Reviewer 2	The document for 6PPD follows EPA's current aquatic life criteria framework, which is organized in a similar manner to EPA 's ecological risk assessment guidance. The organization and construction of this document is easy to follow, and the writing is clearly presented for the most part. However, the document has several grammatical and typographical errors which should be corrected using a thorough editorial review before finalizing.

2.1. Clarity of Document as it Relates to Assessing the Risk of 6PPD to Aquatic-Life	
Reviewer	**Comments**
	This document summarizes what is known about the chemistry, fate, and transport properties of 6PPD in the environment. The document limits this discussion to freshwater as opposed to salt water as well, which is due to the paucity of data regarding fate and transport of 6PPD in saltwater. While the document later explains the lack of marine toxicity data, perhaps EPA should also acknowledge the current lack of fate and transport information in marine environments as well. This document mentions tire wear and wet weather runoff from roads as a major source of 6PPD in surface waters. There has been a fair amount of research in the past seven or eight years regarding fate and transport of 6PPD in surface waters and some of those data are coincident with coho salmon pre-spawn mortality in the Pacific Northwest. EPA should consider incorporating a brief summary of relevant publications in this regard as some of those data may help to support EPA's SV for 6PPD. I included some relevant citations of which I am aware below.
Reviewer 3	This report has high overall clarity and good construction for reporting the results of previous studies regarding the toxic risk of 6PPD to multiple diverse taxonomic groups. Some of the reported data appear redundant. For example, Table 3-3. Ranked Freshwater Genus Mean Acute Values and Figure 3-1. Ranked Freshwater Acute 6PPD GMAVs Fulfilling the Acute Family MDR seem to be reporting the same results. This also appears to be true for Table 3-5 and Figure 3-2. Overall, I have no problem with the presentation of this report with regard to assessing the risk of 6PPD to aquatic life.

2.2 Please comment on the technical approach used to derive the draft screening values presented in EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater.

2.2.a. Is the technical approach used to derive the screening values logical?

2.2.b. Does the science support the conclusions?

2.2.c. Is it consistent with the protection of aquatic life?

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
Reviewer 1	2.2.a. The technical approach generally follows EPA's established procedures for developing a species sensitivity distribution to derive a water quality criterion. However, due to the paucity of acute and especially chronic freshwater toxicity data available at this time, some modifications or "relaxing" of these procedures were employed to derive a draft screening value. Thus, I agree with the decision to use selected qualitative data from studies that did not meet all requirements. Given the current major research focus worldwide on investigating 6PPD and related TWP toxicants, no doubt there will soon

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
	be more data available to further develop water quality criteria for 6PPD (and 6PPD-quinone). 2.2.b. I have a major comment related to this question. The decision to use the Japan Ministry of the Environment (2019) acute toxicity test in medaka is questionable in my opinion. Few details are available on experimental design, including 6PPD purity, use of solvent control, nominal exposure concentrations, routine water quality parameters, or source/age of animals. Was this a static test over 96 hours? What was the loading density of animals? What were the ammonia levels? I do not think that it can be "assumed", as stated in the EPA document, that since Japan is an OECD member that this study was conducted with sufficient rigor. Perhaps additional information can be obtained from this lab to clarify the lack of experimental details? This is important because the draft 6PPD screening value is based largely on the apparent sensitivity of medaka, which is not consistent with all other freshwater species shown in Figure 3-1 (note log scale on x-axis). Note: in the description of this medaka study (page 20, section 3.1.1.1) it states "source of daphnids", is this a typo or were daphnids used as food? Also in this section (last sentence), it states that this value was acceptable for qualitative use, but in Table 3-2 it indicates quantitative use? In summary, given the known labile nature of 6PPD in solution, especially with respect to its transformation to 6PPD-quinone, I think there are too many unknowns in this medaka study to allow it to be used as the driver of the acute 6PPD screening level. However, despite my cautionary opinion above, the study by Hiko et al. (2021) observed 80% mortality in medaka exposed for 96 hours to 107 ug 6PPD/L (measured; only single exposure concentration used), which does provide support for the notion that medaka are very sensitive to 6PPD. Although there were inadequate data available to derive a freshwater 6PPD screening value for chronic exposures, the EPA's decision to consider the Japan Ministry of the Environment (2019) chronic toxicity test using medaka is also fraught with uncertainty in my opinion, for the same reasons listed above. 2.2.c. Given my opinion above on the suitability of the medaka studies, if the Japan Ministry of the Environment (2019) acute toxicity study in medaka was excluded then it appears the most sensitive species would be rare minnow, and the FAV/2 used to derive the screening level would be somewhat greater that the proposed value of 8.3 ug/L. Thus, I do believe that the proposed screening value of 8.3 ug/L would be protective of aquatic life. Although this value may be overly conservative, given the lack of 6PPD freshwater toxicity data available at this time it would be prudent to be precautionary.
Reviewer 2	2.2.a. The technical approach used to derive the screening value (SV) for 6PPD follows EPA's guidelines for deriving aquatic life criteria in general. The document discusses modifications to the guidelines in terms of minimum taxa data requirements, test duration, and some other departures from standard test protocols in terms of what data are considered acceptable or unacceptable to use quantitatively to derive an SV.

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
	Given the fairly low persistence of 6PPD in water exposed to oxygen, and the few toxicity test data currently available that enable a statistically derived point estimate endpoint (for example LC or EC50), the modifications employed are scientifically justified. Specifically, an LC or EC50 based on a test duration greater than or equal to 24 hours rather than 48 or 96 hours depending on the species, should be acceptable given the chemical properties of 6PPD and observed toxicity responses in laboratory exposures. It may be useful to incorporate what is known regarding time to death or immobility based on test results to further support the use of 24-hour test endpoints to derive an acute SV. 2.2.b. The science presented supports the SV for 6PPD given that it is not a national criterion for reasons discussed in the document and it is clearly based on a limited data set (also discussed in the document). A caution that should be even further highlighted in this document is that the significance of 6PPD in terms of aquatic life risk has only recently come to light and there are many ongoing studies that are likely to provide more data with which to refine the SV or an eventual aquatic life criterion. 2.2.c. The technical approach used to derive the SV for 6PPD appears to be consistent with the protection of aquatic life based on the test data currently available; that is, Coho salmon appear to be the most sensitive species of those tested thus far. Therefore, protecting Coho salmon from acute effects with a safety margin provided by EPA's acute criterion derivation procedure used in this document, will hopefully protect aquatic life overall. However, relatively few species have been tested thus far, although that is likely to change over the next few years given worldwide attention now on 6PPD and 6PPD-q. The document should make it even clearer that the SV is based on data obtained thus far and may have high uncertainty.
Reviewer 3	The U.S. Environmental Protection Agency (EPA) developed the preliminary draft screening value for 6PPD in accordance with Section 304(a) of the Clean Water Act (CWA). EPA has developed the 6PPD screening value for sensitive aquatic life in a manner that is generally consistent with methods described in EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" U.S.EPA (1985) and EPA's OSCPP's Ecological Effects Test Guidelines (U.S.EPA 2016a) given the methodological limitations of some of the older studies. Thus, some of the methodology may not have been ideal for determining toxicity. For example, in the Monsanto Co. 1979 study, the concentrations used for a 28-d flow thru proportional diluter test were prepared as a simple arithmetic progression doubling the five concentrations from 0.066 to 1 mg/L of PPD. No mention was made of a using a range-finding test to come up with these concentrations. If this was indeed the range-finding test then the concentrations should have been prepared as a logarithmic progression. This is also true of the Monsanto Co. (1984) study. I believe for the more recent study they should have employed the correct array if concentrations. It should be noted that other studies (e.g. Peng 2022), correctly employed range-finding tests and eventually settled on a similar concentration array as Monsanto 1979 and 1984. However, they then calculated the LC_{50} using nominal concentration instead of the actual measured concentrations which were available. This unfortunately increases the uncertainty in predicting

2.2. The Technical Approach Used to Derive the Draft Screening Values	
Reviewer	**Comments**
	toxicity in natural systems. However, even given the stated reservations, the technical approach was appropriate and useful and yielded important information. 2.2.a. In arriving at preliminary draft screening value for acute exposures of 6PPD in freshwater, EPA attempted to review and consider all relevant acute toxicity test data. The goal here was to identify all data from acceptable tests that met data quality standards and thus determine acceptable data meeting the minimum data requirements (MDRs) as outlined in EPA's 1985 Guidelines (U.S.EPA 1985). The MDRs described in Section 2.1.1 were not met for acute freshwater criteria derivation. Acceptable studies of aquatic algae and vascular plants were also not available. Consequently, national 304(a) ambient water quality criteria for the protection of aquatic life could not be derived for 6PPD. The authors were able to derive a protective acute screening value for 6PPD in freshwater by dividing the Final Acute Value (FAV) by two to obtain a concentration yielding a minimal effects acute screening value. Based on the above, the FAV/2, which is the freshwater acute water column screening value, is **8.3 µg/L 6PPD**. This assessment quantifies the acute toxicity of 6PPD to aquatic organisms to protect aquatic life in freshwater. The 6PPD screening value is expected to be protective of most sensitive aquatic organisms in the community and is derived to be protective of aquatic life designated uses. It must be noted that this preliminary draft screening value for 6PPD is based on fewer empirical data than an aquatic life criterion would use and therefore has greater inherent uncertainty regarding environmental risk assessment. 2.2.b. Overall, the science does support the conclusions (derivation of a useful freshwater screening value). Because the studies evaluated are by multiple laboratories over a wide time frame (as early as 1977 and as recent as 2022) there is an unavoidable unevenness in toxicity testing methodology, presentation of results, and interpretation. There were also some issues with chemical analyses, specifically the level of purity of N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine on Medaka, N-(1,3-dimethylbutyl)-N'-phenyl-1,4-benzenediamine and N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine on the cladoceran *Daphnia magna*, and N-(1,3-Dimethylbutyl)-N'-Phenyl-P-Phenylenediamine on the Fathead Minnow *Pimephales promelas* were all unknown. Nevertheless, the authors appeared to produce a credible product based on the data available to them. 2.2.c. All of the studies included in this report (after excluding numerous studies with insufficient useful data) were generally following multiple EPA guidelines published between 1975 and 2022. Thus, I am reasonably confident that (given the well-documented limitations of these guidelines) all except the most sensitive aquatic life taxa will be afforded a reasonable protection associated with exposure to 6PPD.

2.3 Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD document.

2.3.a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

2.3.b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

2.3.c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

2.3. The Toxicity Data used to Derive the Screening Values	
Reviewer	**Comments**
Reviewer 1	2.3.a. Despite my concerns provided above in detail, I do think EPA made the most out of the limited data available at this time. I agree that data were insufficient to currently derive an acute freshwater criterion, and agree with first developing a preliminary draft screening value for 6PPD. I also agree that data are not sufficient to derive a chronic screening value for freshwater. Given the lack of saltwater/estuarine species data, I also concur with the inability to derive a screening level for these ecosystems. However, it may be worth noting here that in the wild, the Japanese medaka is a euryhaline teleost that inhabits tidal/estuarine ecosystems in Asia so may be somewhat relevant to saltwater/brackish environments. Despite this point, I do realize that there are no studies that used saline exposure water. 2.3.b. As stated above, I think EPA made the most of out of the limited aquatic toxicity dataset and appropriately included/excluded data to derive the screening value, with the exception of my concerns on utilizing the medaka acute toxicity study (Japan Ministry of the Environment 2019). I agree with (i) using nominal exposure concentrations in studies that did not quantitate 6PPD, (ii) using studies with greater than or equal to 24-hour exposure durations, particularly due to the environmental realism of such short-term exposure scenarios in the wild, and (iii) considering non-native aquatic species such as medaka and zebrafish in derivation of this screening level. I think the study by Prosser et al. (2017c) in early life stage fathead minnow should be reconsidered. This well-conducted, peer-reviewed study was excluded due to use of 6PPD-spiked sediment in exposure vessels, thus not following EPA's data inclusion rules. However, fathead minnow embryo-larval stages were exposed aqueously in isolated "egg cups" suspended in the water column (i.e., not directly in contact with sediment), and aqueous 6PPD was measured in this 21-day study. Thus, I think the study warrants further consideration in the derivation of screening values for 6PPD. I am concerned about the water quality reported in the Monsanto (1979) study in fathead minnow (section 3.1.1.1.7). Specifically, the reported ammonia concentration of 0.9 mg/L is near lethal levels for fish at pH7.8. I suggest double-checking this value, and if correct then consider not employing this study for quantitative use. 2.3.c. I am not aware of any further data on 6PPD aquatic toxicity.

2.3. The Toxicity Data used to Derive the Screening Values	
Reviewer	**Comments**
Reviewer 2	2.3.a. The toxicity data obtained and discussed in this document appears as comprehensive as EPA can be at this time. The document is clear regarding the limitations of the data and it may be worth adding that data are currently limited because it is only very recently that the significance of 6PPD has been identified in terms of aquatic life risk. 2.3.b. The data selected or not selected to derive the SV for 6PPD appears scientifically justified and consistent with the goal of protecting aquatic life. As noted in response to charge question 1, given the low persistence of 6PPD and the few test data available, endpoints based on > 24 hour exposures, and/or higher fish loading per test chamber are acceptable to use in this case given other test acceptance rationale applied in the document (e.g., dissolved oxygen and ammonia concentration were acceptably low and control survival met EPA's test acceptability criterion for acute tests despite higher fish loading). 2.3.c. This reviewer is not aware of other toxicity test data that have been published and would satisfy basic requirements of acceptability according to EPA's aquatic life criteria guidelines- for example, test data based on organism exposure to the chemical of interest only and not a field study for example, where other stressors, chemical and otherwise, may be present.
Reviewer 3	As mentioned above, unevenness in testing protocols, toxicity data collection and interpretation of results over a 45-year span makes it challenging to coalesce the data into screening values. I believe this attempt has been a reasonable effort to address this challenge with the available data and think that the risk associated with this type of meta-analysis is acceptable for an initial pass at producing guidelines for controlling 6PPD planned and unplanned releases such as used automobile tire reefs and storm sewer associated settling ponds and wetland mitigation. 2.3.a. The authors attempted to utilize all available historical studies in this meta-analysis. However, overall aquatic life toxicity data for 6PPD were limited, especially with regard to freshwater chronic testing for which the data sets were extremely limited. This resulted in an inability to develop a freshwater chronic screening value. Additionally acute and chronic estuarine/marine data were completely unavailable. While certainly not a complete development of risks of 6PPD to sensitive aquatic life, it is a reasonable early attempt to do so. I believe, using currently available EPA Testing facilities in Region 6 (Houston) or Region 5 (Cincinnati), or independent EPA-Certified laboratories such as the Ecotoxicology Research Facility in Jonesboro, AR, the necessary data for a well-supported environmental risk analysis of 6PPD could be completed in 6 – 9 months, perhaps in a round-robin format. This would render the current meta-analysis mostly obsolete. This approach requires slicing thru a significant amount of red tape but it could be done if a suitable project coordinator is identified.

2.3. The Toxicity Data used to Derive the Screening Values	
Reviewer	**Comments**
	2.3.b. The authors correctly concluded that the chronic data set for 6PPD was much more limited in scope and content in comparison to acute data. Because of the limited chronic data available for 6PPD, a chronic screening value for aquatic life in freshwater was not derivable. While the current acute 6PPD data does not support the complete derivation of an acute water column criterion in freshwater, the dataset does enable the calculation of an acute screening value for freshwaters. Thus, I believe the available data were appropriately utilized. 2.3.c. I do not believe other relative data exists that should have been included in the evaluation of 6PPD toxicity. The authors appear to have completed an exhaustive literature review which identified most/all of the previous studies on this compound. These were all carefully evaluated using a specific set of acceptability criteria to determine if the results should be included in the derivation of the final screening value.

2.4 Are the derived screening values appropriately protective of sensitive aquatic life?

2.4. The Derived Screening Values' Protectiveness to Sensitive Aquatic Life	
Reviewer	**Comments**
Reviewer 1	Yes, despite the relative lack of 6PPD toxicity data in freshwater, I do believe the proposed screening values would be protective of aquatic life. Some final thoughts for consideration: Given the labile nature of 6PPD (half-life in the environment of less than one day), its known susceptibility to hydrolysis and photolysis, and particularly a study that reported detection of 6PPD-quinone 4 hours after spiking water with 6PPD (Hiki et al. 2021; cited in the EPA document), it seems to me that this creates a large uncertainty in evaluating aquatic toxicity testing of this specific chemical for the following reasons: (i) if purity is reported as 95-98%, what is the remaining 2-5%? Could some of this be the more toxic 6PPD-quinone? (ii) if purity is not reported, how can it ever be determined whether the results truly represent actual 6PPD toxicity? (iii) in 96-hour static-renewal experiments with replacement every 24 or 48 hours (or not at all) how can it ever be determined whether the results truly represent actual 6PPD toxicity? My point here is related to implementation of the 6PPD screening level. Is a 6PPD screening level needed? Would valuable resources be wasted measuring 6PPD in aquatic systems when the real culprit is 6PPD-quinone (or potentially other degradation products of 6PPD)?
Reviewer 2	The derived SV for 6PPD appears appropriate given the toxicity data available currently. However, the fact that that one of its degradates, 6PPD-q, is clearly more toxic than 6PPD, and 6PPD-q occurs under ordinary aerobic conditions in surface waters (albeit in low concentrations typically), protection of aquatic life may or may not be assured based on the proposed 6PPD SV. Other than setting the SV for 6PPD equal to the SV for 6PPD-q (or some other concentration that ensures the SV for 6PPD-q is not exceeded under typical surface

2.4. The Derived Screening Values' Protectiveness to Sensitive Aquatic Life	
Reviewer	**Comments**
	water conditions), it is not certain that meeting the SV for 6PPD will in fact protect aquatic life due to toxic concentrations of its degradate 6PPD-q.
Reviewer 3	The US EPA developed the final screening value following the general approach outlined in the EPA's "*Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (U.S.EPA 1985). Additional toxicity data (especially with taxa that are missing) and repeated toxicity studies for taxa using current methods for data generation are needed to better understand the toxicity of 6PPD and to derive national ambient water quality criteria to protect aquatic life to 6PPD.

3.0 ADDITIONAL REVIEWER COMMENTS

Reviewer	Comments
Reviewer 1	See minor edits provided in the draft document.
Reviewer 2	Additional literature regarding fate and transport for EPA's consideration: • Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. 2020. Tire and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. *Science of The Total Environment*, 137823. • French, B.F., D. H. Baldwin, J. Cameron, J. Prat, K. King, J. W. Davis, J. K. McIntyre, and N. L. Scholz. 2022. *Environmental Science & Technology Letters* 9: 733-738 • McIntyre, J.F., J. Prat, J. Cameron, J. Wetzel, E. Murdock, K.T. Peter, Z. Tian, C. Mackenzie, J. Lundin, J. D. Stark, K. King, J.W. Davis, E.P. Kolodziej, and N. L. Scholz. 2021. Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon. *Environ. Sci. Technol*. 55: 11767−11774 • Peter, K.T., F. Hou, Z. Tian, C. Wu, M. Goehring, F. Liu, and E. P. Kolodziej. 2020. More Than a First Flush: Urban Creek Storm Hydrographs Demonstrate Broad Contaminant Pollutographs. *Environ. Sci. Technol*., 54: 6152–6165. • Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., & Thorsten, R. 2022. Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. *Water Research, 212*. • Unice, K. M., Bare, J. L., Kreider, M. L., & Panko, J. M. 2015. Experimental methodology for assessing the environmental fate of organic chemicals in polymer matrices using

Reviewer	Comments
	column leaching studies and OECD 308 water/sediment systems: Application to tire and road wear particles. *The Science of the Total Environment* 533: 476-487.
Reviewer 3	(Note: only publications not already listed in the two reports are listed here) • J. A. Spromberg, N. L. Scholz, Estimating the future decline of wild coho salmon populations resulting from early spawner die-offs in urbanizing watersheds of the Pacific Northwest, USA. *Integr. Environ. Assess. Manag.* **7**, 648–656 (2011). • K. T. Peter, Z. Tian, C. Wu, P. Lin, S. White, B. Du, J. K. McIntyre, N. L. Scholz, E. P. Kolodziej, Using high-resolution mass spectrometry to identify organic contaminants linked to urban stormwater mortality syndrome in coho salmon. *Environ. Sci. Technol.* **52**, 10317–10327 (2018). • Tian, Z., H. Zhao, K. T. Peter, M. Gonzalez, J. Wetzel, C. Wu, X. Hu, J. Prat, E. Mudrock and R. Hettinger. 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science. 371(6525): 185-189.

APPENDIX A

CHARGE TO REVIEWERS

Technical Charge to External Peer Reviewers

Contract GSA GS-00F-079CA; BPA #68HERH23A0019
Call Order 68HERH23F0365 (ERG Call Order #04)
October 2023

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater

BACKGROUND

The purpose of EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater and Preliminary Draft Sensitive Salmonid Screening Value for Acute Exposures to 6PPD-Quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) in Freshwater is to provide EPA's scientific rationale for the draft screening values for 6PPD and 6PPD-quinone focused on the protection of aquatic life. The criteria are designed to solely protect sensitive aquatic life and is based on the best available data and best professional scientific judgements on the toxicological effects of 6PPD and 6PPD-quinone to aquatic life.

N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine (6PPD) is an additive to vehicle tire rubber, where it functions to protect rubber from reactions with ozone and oxygen, which can lead to degradation and cracking. Recent research has determined the ozonation of (6PPD) produces the reaction product 6PPD-quinone (N-(1,3-Dimethylbutyl)-N'-phenyl-p-phenylenediamine quinone) through hydrolysis and photodegradation. 6PPD-quinone recently has been shown to present an environmental threat based on its toxicity to salmon, where it has been identified as the causal agent in urban runoff mortality syndrome (URMS) observed in the Puget Sound area of Washington state.

Relatively limited 6PPD and 6PPD-quinone toxicity studies have been conducted on various aquatic taxa, including numerous species of fish and invertebrates, indicating that exposure to 6PPD and 6PPD-quinone through the aquatic environment causes population level effects, particularly mortality to acute exposures across aquatic life in the case for 6PPD and to fish such as salmonids, which appear to be sensitive to 6PPD-quinone exposures in particular. In these drafts, EPA provides support for and outlines the derivation of screening values for 6PPD and 6PPD-quinone that would be protective of sensitive aquatic life.

CHARGE QUESTIONS

1) Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD to aquatic life.

2) Please comment on the technical approach used to derive the draft screening vales presented in *EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater*.

    a. Is the technical approach used to derive the screening values logical?
    b. Does the science support the conclusions?
    c. Is it consistent with the protection of aquatic life?

3) Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD document.

   a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?
   b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?
   c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

4) Are the derived screening values appropriately protective of sensitive aquatic life?

APPENDIX B

INDIVIDUAL REVIEWER COMMENTS

COMMENTS SUBMITTED BY

REVIEWER 1

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater

CHARGE QUESTIONS

1. **Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD to aquatic life.**

The document is generally well written and flows logically. The background summary of environmental fate and distribution of 6PPD was good. The explanation of how EPA derives water quality criteria was clear. I have provided a marked-up version with minor editorial suggestions and comments.

There are several inconsistencies in the descriptions of experimental procedures when summarizing the various studies. The document must be consistent and specific when describing the control treatments among studies. In all instances, specify "solvent control" or "solvent vehicle control" when such a control was employed, and when known state the solvent concentration occurring in the actual exposure and control vessels as a percentage volume/volume. For example, I was confused on page 44 lines 5-7 ("No mortality was observed in the control or treatment concentration. Mortality in the control was 5%, and mortality in the test concentration was 100%"). I am assuming that "treatment concentration" refers to the solvent control?

In addition, please specify what is meant by a negative control. Is this water-only, without solvent added?

In the study descriptions (initial sentence of each) be consistent and always state whether 6PPD was measured or unmeasured.

2. **Please comment on the technical approach used to derive the draft screening values presented in EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater.**

   a. **Is the technical approach used to derive the screening values logical?**

      The technical approach generally follows EPA's established procedures for developing a species sensitivity distribution to derive a water quality criterion. However, due to the paucity of acute and especially chronic freshwater toxicity data available at this time, some modifications or "relaxing" of these procedures were employed to derive a draft screening value. Thus, I agree with the decision to use selected qualitative data from studies that did not meet all requirements. Given the current major research focus worldwide on investigating 6PPD and related TWP toxicants, no doubt there will soon be more data available to further develop water quality criteria for 6PPD (and 6PPD-quinone).

   b. **Does the science support the conclusions?**

      I have a major comment related to this question. The decision to use the Japan Ministry of the Environment (2019) acute toxicity test in medaka is questionable in my opinion. Few details are available on experimental design, including 6PPD purity, use of solvent control, nominal exposure concentrations, routine water quality parameters, or source/age of animals. Was this a static test over 96 hours? What was the loading density of animals? What were the ammonia levels? I do not think that it can be "assumed", as stated in the EPA document, that since Japan is an OECD member

that this study was conducted with sufficient rigor. Perhaps additional information can be obtained from this lab to clarify the lack of experimental details? This is important because the draft 6PPD screening value is based largely on the apparent sensitivity of medaka, which is not consistent with all other freshwater species shown in Figure 3-1 (note log scale on x-axis). Note: in the description of this medaka study (page 20, section 3.1.1.1) it states "source of daphnids", is this a typo or were daphnids used as food? Also in this section (last sentence), it states that this value was acceptable for qualitative use, but in Table 3-2 it indicates quantitative use?

In summary, given the known labile nature of 6PPD in solution, especially with respect to its transformation to 6PPD-quinone, I think there are too many unknowns in this medaka study to allow it to be used as the driver of the acute 6PPD screening level. However, despite my cautionary opinion above, the study by Hiko et al. (2021) observed 80% mortality in medaka exposed for 96 hours to 107 ug 6PPD/L (measured; only single exposure concentration used), which does provide support for the notion that medaka are very sensitive to 6PPD.

Although there were inadequate data available to derive a freshwater 6PPD screening value for chronic exposures, the EPA's decision to consider the Japan Ministry of the Environment (2019) chronic toxicity test using medaka is also fraught with uncertainty in my opinion, for the same reasons listed above.

c. Is it consistent with the protection of aquatic life?

Given my opinion above on the suitability of the medaka studies, if the Japan Ministry of the Environment (2019) acute toxicity study in medaka was excluded then it appears the most sensitive species would be rare minnow, and the FAV/2 used to derive the screening level would be somewhat greater that the proposed value of 8.3 ug/L. Thus, I do believe that the proposed screening value of 8.3 ug/L would be protective of aquatic life. Although this value may be overly conservative, given the lack of 6PPD freshwater toxicity data available at this time it would be prudent to be precautionary.

3. Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD document.

a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

Despite my concerns provided above in detail, I do think EPA made the most out of the limited data available at this time. I agree that data were insufficient to currently derive an acute freshwater criterion, and agree with first developing a preliminary draft screening value for 6PPD. I also agree that data are not sufficient to derive a chronic screening value for freshwater. Given the lack of saltwater/estuarine species data, I also concur with the inability to derive a screening level for these ecosystems. However, it may be worth noting here that in the wild, the Japanese medaka is a euryhaline teleost that inhabits tidal/estuarine ecosystems in Asia so may be somewhat relevant to saltwater/brackish environments. Despite this point, I do realize that there are no studies that used saline exposure water.

b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

As stated above, I think EPA made the most of out of the limited aquatic toxicity dataset and appropriately included/excluded data to derive the screening value, with the exception of my concerns on utilizing the medaka acute toxicity study (Japan Ministry of the Environment 2019). I agree with (i) using nominal exposure concentrations in studies that did not quantitate 6PPD, (ii) using studies with greater than or equal to 24-hour exposure durations, particularly due to the environmental realism of such short-term exposure scenarios in the wild, and (iii) considering non-native aquatic species such as medaka and zebrafish in derivation of this screening level.

I think the study by Prosser et al. (2017c) in early life stage fathead minnow should be reconsidered. This well-conducted, peer-reviewed study was excluded due to use of 6PPD-spiked sediment in exposure vessels, thus not following EPA's data inclusion rules. However, fathead minnow embryo-larval stages were exposed aqueously in isolated "egg cups" suspended in the water column (i.e., not directly in contact with sediment), and aqueous 6PPD was measured in this 21-day study. Thus, I think the study warrants further consideration in the derivation of screening values for 6PPD.

I am concerned about the water quality reported in the Monsanto (1979) study in fathead minnow (section 3.1.1.1.7). Specifically, the reported ammonia concentration of 0.9 mg/L is near lethal levels for fish at pH7.8. I suggest double-checking this value, and if correct then consider not employing this study for quantitative use.

c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

I am not aware of any further data on 6PPD aquatic toxicity.

4. Are the derived screening values appropriately protective of sensitive aquatic life?

Yes, despite the relative lack of 6PPD toxicity data in freshwater, I do believe the proposed screening values would be protective of aquatic life.

Some final thoughts for consideration: Given the labile nature of 6PPD (half-life in the environment of less than one day), its known susceptibility to hydrolysis and photolysis, and particularly a study that reported detection of 6PPD-quinone 4 hours after spiking water with 6PPD (Hiki et al. 2021; cited in the EPA document), it seems to me that this creates a large uncertainty in evaluating aquatic toxicity testing of this specific chemical for the following reasons: (i) if purity is reported as 95-98%, what is the remaining 2-5%? Could some of this be the more toxic 6PPD-quinone? (ii) if purity is not reported, how can it ever be determined whether the results truly represent actual 6PPD toxicity? (iii) in 96-hour static-renewal experiments with replacement every 24 or 48 hours (or not at all) how can it ever be determined whether the results truly represent actual 6PPD toxicity? My point here is related to implementation of the 6PPD screening level. Is a 6PPD screening level needed? Would valuable resources be wasted measuring 6PPD in aquatic systems when the real culprit is 6PPD-quinone (or potentially other degradation products of 6PPD)?

COMMENTS SUBMITTED BY

REVIEWER 2

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater

CHARGE QUESTIONS

1. **Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD to aquatic life.**

The document for 6PPD follows EPA's current aquatic life criteria framework, which is organized in a similar manner to EPA 's ecological risk assessment guidance. The organization and construction of this document is easy to follow, and the writing is clearly presented for the most part. However, the document has several grammatical and typographical errors which should be corrected using a thorough editorial review before finalizing.

This document summarizes what is known about the chemistry, fate, and transport properties of 6PPD in the environment. The document limits this discussion to freshwater as opposed to salt water as well, which is due to the paucity of data regarding fate and transport of 6PPD in saltwater. While the document later explains the lack of marine toxicity data, perhaps EPA should also acknowledge the current lack of fate and transport information in marine environments as well.

This document mentions tire wear and wet weather runoff from roads as a major source of 6PPD in surface waters. There has been a fair amount of research in the past seven or eight years regarding fate and transport of 6PPD in surface waters and some of those data are coincident with coho salmon pre-spawn mortality in the Pacific Northwest. EPA should consider incorporating a brief summary of relevant publications in this regard as some of those data may help to support EPA's SV for 6PPD. I included some relevant citations of which I am aware below.

2. **Please comment on the technical approach used to derive the draft screening vales presented in *EPA's Preliminary Draft SV for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater*.**

    a. **Is the technical approach used to derive the SVs logical?**

    The technical approach used to derive the screening value (SV) for 6PPD follows EPA's guidelines for deriving aquatic life criteria in general. The document discusses modifications to the guidelines in terms of minimum taxa data requirements, test duration, and some other departures from standard test protocols in terms of what data are considered acceptable or unacceptable to use quantitatively to derive an SV. Given the fairly low persistence of 6PPD in water exposed to oxygen, and the few toxicity test data currently available that enable a statistically derived point estimate endpoint (for example LC or EC50), the modifications employed are scientifically justified. Specifically, an LC or EC50 based on a test duration greater than or equal to 24 hours rather than 48 or 96 hours depending on the species, should be acceptable given the chemical properties of 6PPD and observed toxicity responses in laboratory exposures. It may be useful to incorporate what is known regarding time to death or immobility based on test results to further support the use of 24-hour test endpoints to derive an acute SV.

b. Does the science support the conclusions?

The science presented supports the SV for 6PPD given that it is not a national criterion for reasons discussed in the document and it is clearly based on a limited data set (also discussed in the document). A caution that should be even further highlighted in this document is that the significance of 6PPD in terms of aquatic life risk has only recently come to light and there are many ongoing studies that are likely to provide more data with which to refine the SV or an eventual aquatic life criterion.

c. Is it consistent with the protection of aquatic life?

The technical approach used to derive the SV for 6PPD appears to be consistent with the protection of aquatic life based on the test data currently available; that is, Coho salmon appear to be the most sensitive species of those tested thus far. Therefore, protecting Coho salmon from acute effects with a safety margin provided by EPA's acute criterion derivation procedure used in this document, will hopefully protect aquatic life overall. However, relatively few species have been tested thus far, although that is likely to change over the next few years given worldwide attention now on 6PPD and 6PPD-q. The document should make it even clearer that the SV is based on data obtained thus far and may have high uncertainty.

3. Please comment on the toxicity data used to derive the SVs presented in the draft 6PPD document.

a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

The toxicity data obtained and discussed in this document appears as comprehensive as EPA can be at this time. The document is clear regarding the limitations of the data and it may be worth adding that data are currently limited because it is only very recently that the significance of 6PPD has been identified in terms of aquatic life risk.

b. Were the data selected and/or excluded from the SVs derivation appropriately utilized?

The data selected or not selected to derive the SV for 6PPD appears scientifically justified and consistent with the goal of protecting aquatic life. As noted in response to charge question 1, given the low persistence of 6PPD and the few test data available, endpoints based on $\geq$ 24 hour exposures, and/or higher fish loading per test chamber are acceptable to use in this case given other test acceptance rationale applied in the document (e.g., dissolved oxygen and ammonia concentration were acceptably low and control survival met EPA's test acceptability criterion for acute tests despite higher fish loading).

c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of SVs.

This reviewer is not aware of other toxicity test data that have been published and would satisfy basic requirements of acceptability according to EPA's aquatic life criteria guidelines- for example, test data based on organism exposure to the chemical of interest only and not a field study for example, where other stressors, chemical and otherwise, may be present.

4. Are the derived SVs appropriately protective of sensitive aquatic life?

The derived SV for 6PPD appears appropriate given the toxicity data available currently. However, the fact that that one of its degradates, 6PPD-q, is clearly more toxic than 6PPD, and 6PPD-q occurs under ordinary aerobic conditions in surface waters (albeit in low concentrations typically), protection of aquatic life may or may not be assured based on the proposed 6PPD SV. Other than setting the SV for 6PPD equal to the SV for 6PPD-q (or some other concentration that ensures the SV for 6PPD-q is not exceeded under typical surface water conditions), it is not certain that meeting the SV for 6PPD will in fact protect aquatic life due to toxic concentrations of its degradate 6PPD-q.

Additional literature regarding fate and transport for EPA's consideration

- Baensch-Baltruschat, B., Kocher, B., Stock, F., & Reifferscheid, G. 2020. Tire and road wear particles (TRWP) - A review of generation, properties, emissions, human health risk, ecotoxicity, and fate in the environment. *Science of The Total Environment*, 137823.
- French, B.F., D. H. Baldwin, J. Cameron, J. Prat, K. King, J. W. Davis, J. K. McIntyre, and N. L. Scholz. 2022. *Environmental Science & Technology Letters* 9: 733-738
- McIntyre, J.F., J. Prat, J. Cameron, J. Wetzel, E. Murdock, K.T. Peter, Z. Tian, C. Mackenzie, J. Lundin, J. D. Stark, K. King, J.W. Davis, E.P. Kolodziej, and N. L. Scholz. 2021. Treading Water: Tire Wear Particle Leachate Recreates an Urban Runoff Mortality Syndrome in Coho but Not Chum Salmon. *Environ. Sci. Technol*. 55: 11767−11774
- Peter, K.T., F. Hou, Z. Tian, C. Wu, M. Goehring, F. Liu, and E. P. Kolodziej. 2020. More Than a First Flush: Urban Creek Storm Hydrographs Demonstrate Broad Contaminant Pollutographs. *Environ. Sci. Technol*., 54: 6152–6165.
- Seiwert, B., Nihemaiti, M., Troussier, M., Weyrauch, S., & Thorsten, R. 2022. Abiotic oxidative transformation of 6-PPD and 6-PPD quinone from tires and occurrence of their products in snow from urban roads and in municipal wastewater. *Water Research, 212*.
- Unice, K. M., Bare, J. L., Kreider, M. L., & Panko, J. M. 2015. Experimental methodology for assessing the environmental fate of organic chemicals in polymer matrices using column leaching studies and OECD 308 water/sediment systems: Application to tire and road wear particles. *The Science of the Total Environment* 533: 476-487.

COMMENTS SUBMITTED BY

REVIEWER 3

External Peer Review of EPA's Draft Aquatic Life Screening Values for N-(1,3-dimethylbutyl)-N'-phenyl-p-phenylenediamine (6PPD) in Freshwater

CHARGE QUESTIONS

1. **Please comment on the overall clarity of the documents and construction as it relates to assessing the risk of 6PPD to aquatic life.**

This report has high overall clarity and good construction for reporting the results of previous studies regarding the toxic risk of 6PPD to multiple diverse taxonomic groups. Some of the reported data appear redundant. For example, Table 3-3. Ranked Freshwater Genus Mean Acute Values and Figure 3-1. Ranked Freshwater Acute 6PPD GMAVs Fulfilling the Acute Family MDR seem to be reporting the same results. This also appears to be true for Table 3-5 and Figure 3-2. Overall, I have no problem with the presentation of this report with regard to assessing the risk of 6PPD to aquatic life.

2. **Please comment on the technical approach used to derive the draft screening vales presented in EPA's Preliminary Draft Screening Value for Acute Exposures to 6PPD (N-(1,3-dimethylbutyl)-N' -phenyl-p-phenylenediamine) in Freshwater.**

The U.S. Environmental Protection Agency (EPA) developed the preliminary draft screening value for 6PPD in accordance with Section 304(a) of the Clean Water Act (CWA). EPA has developed the 6PPD screening value for sensitive aquatic life in a manner that is generally consistent with methods described in EPA's "*Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*" U.S.EPA (1985) and EPA's OSCPP's Ecological Effects Test Guidelines (U.S.EPA 2016a) given the methodological limitations of some of the older studies. Thus, some of the methodology may not have been ideal for determining toxicity. For example, in the Monsanto Co. 1979 study, the concentrations used for a 28-d flow thru proportional diluter test were prepared as a simple arithmetic progression doubling the five concentrations from 0.066 to 1 mg/L of PPD. No mention was made of a using a range-finding test to come up with these concentrations. If this was indeed the range-finding test then the concentrations should have been prepared as a logarithmic progression. This is also true of the Monsanto Co. (1984) study. I believe for the more recent study they should have employed the correct array if concentrations. It should be noted that other studies (e.g. Peng 2022), correctly employed range-finding tests and eventually settled on a similar concentration array as Monsanto 1979 and 1984. However, they then calculated the LC_{50} using nominal concentration instead of the actual measured concentrations which were available. This unfortunately increases the uncertainty in predicting toxicity in natural systems. However, even given the stated reservations, the technical approach was appropriate and useful and yielded important information.

   a. **Is the technical approach used to derive the screening values logical?**

      In arriving at preliminary draft screening value for acute exposures of 6PPD in freshwater, EPA attempted to review and consider all relevant acute toxicity test data. The goal here was to identify all data from acceptable tests that met data quality standards and thus determine acceptable data meeting the minimum data requirements (MDRs) as outlined in EPA's 1985 Guidelines (U.S.EPA 1985). The MDRs described in Section 2.1.1 were not met for acute freshwater criteria derivation. Acceptable studies of aquatic algae and vascular plants were also not available. Consequently,

national 304(a) ambient water quality criteria for the protection of aquatic life could not be derived for 6PPD.

The authors were able to derive a protective acute screening value for 6PPD in freshwater by dividing the Final Acute Value (FAV) by two to obtain a concentration yielding a minimal effects acute screening value. Based on the above, the FAV/2, which is the freshwater acute water column screening value, is **8.3 µg/L 6PPD.** This assessment quantifies the acute toxicity of 6PPD to aquatic organisms to protect aquatic life in freshwater. The 6PPD screening value is expected to be protective of most sensitive aquatic organisms in the community and is derived to be protective of aquatic life designated uses. It must be noted that this preliminary draft screening value for 6PPD is based on fewer empirical data than an aquatic life criterion would use and therefore has greater inherent uncertainty regarding environmental risk assessment.

b. Does the science support the conclusions?

Overall, the science does support the conclusions (derivation of a useful freshwater screening value). Because the studies evaluated are by multiple laboratories over a wide time frame (as early as 1977 and as recent as 2022) there is an unavoidable unevenness in toxicity testing methodology, presentation of results, and interpretation. There were also some issues with chemical analyses, specifically the level of purity of N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine on Medaka, N-(1,3-dimethylbutyl)-N'-phenyl-1,4-benzenediamine and N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylenediamine on the cladoceran *Daphnia magna*, and N-(1,3-Dimethylbutyl)-N'-Phenyl-P-Phenylenediamine on the Fathead Minnow *Pimephales promelas* were all unknown. Nevertheless, the authors appeared to produce a credible product based on the data available to them.

c. Is it consistent with the protection of aquatic life?

All of the studies included in this report (after excluding numerous studies with insufficient useful data) were generally following multiple EPA guidelines published between 1975 and 2022. Thus, I am reasonably confident that (given the well-documented limitations of these guidelines) all except the most sensitive aquatic life taxa will be afforded a reasonable protection associated with exposure to 6PPD.

3. Please comment on the toxicity data used to derive the screening values presented in the draft 6PPD document.

As mentioned above, unevenness in testing protocols, toxicity data collection and interpretation of results over a 45-year span makes it challenging to coalesce the data into screening values. I believe this attempt has been a reasonable effort to address this challenge with the available data and think that the risk associated with this type of meta-analysis is acceptable for an initial pass at producing guidelines for controlling 6PPD planned and unplanned releases such as used automobile tire reefs and storm sewer associated settling ponds and wetland mitigation.

a. Were the data adequately used and sufficiently comprehensive to represent risks to sensitive aquatic life?

The authors attempted to utilize all available historical studies in this meta-analysis. However, overall aquatic life toxicity data for 6PPD were limited, especially with regard to freshwater chronic testing for which the data sets were extremely limited. This resulted in an inability to develop a freshwater chronic screening value. Additionally acute and chronic estuarine/marine data were completely unavailable. While certainly not a complete development of risks of 6PPD to sensitive aquatic life, it is a reasonable early attempt to do so.

I believe, using currently available EPA Testing facilities in Region 6 (Houston) or Region 5 (Cincinnati), or independent EPA-Certified laboratories such as the Ecotoxicology Research Facility in Jonesboro, AR, the necessary data for a well-supported environmental risk analysis of 6PPD could be completed in 6 – 9 months, perhaps in a round-robin format. This would render the current meta-analysis mostly obsolete. This approach requires slicing thru a significant amount of red tape but it could be done if a suitable project coordinator is identified.

b. Were the data selected and/or excluded from the screening values derivation appropriately utilized?

The authors correctly concluded that the chronic data set for 6PPD was much more limited in scope and content in comparison to acute data. Because of the limited chronic data available for 6PPD, a chronic screening value for aquatic life in freshwater was not derivable.

While the current acute 6PPD data does not support the complete derivation of an acute water column criterion in freshwater, the dataset does enable the calculation of an acute screening value for freshwaters. Thus, I believe the available data were appropriately utilized.

c. Are there relevant data that you are aware of that should be included? If so, please provide for derivation of screening values.

I do not believe other relative data exists that should have been included in the evaluation of 6PPD toxicity. The authors appear to have completed an exhaustive literature review which identified most/all of the previous studies on this compound. These were all carefully evaluated using a specific set of acceptability criteria to determine if the results should be included in the derivation of the final screening value.

4. Are the derived screening values appropriately protective of sensitive aquatic life?

The US EPA developed the final screening value following the general approach outlined in the EPA's "*Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses"* (U.S.EPA 1985). Additional toxicity data (especially with taxa that are missing) and repeated toxicity studies for taxa using current methods for data generation are needed to better understand the toxicity of 6PPD and to derive national ambient water quality criteria to protect aquatic life to 6PPD.

Literature cited (Note: only publications not already listed in the two reports are listed here)

- J. A. Spromberg, N. L. Scholz, Estimating the future decline of wild coho salmon populations resulting from early spawner die-offs in urbanizing watersheds of the Pacific Northwest, USA. *Integr. Environ. Assess. Manag.* **7**, 648–656 (2011).
- K. T. Peter, Z. Tian, C. Wu, P. Lin, S. White, B. Du, J. K. McIntyre, N. L. Scholz, E. P. Kolodziej, Using high-resolution mass spectrometry to identify organic contaminants linked to urban stormwater mortality syndrome in coho salmon. *Environ. Sci. Technol.* **52**, 10317–10327 (2018).
- Tian, Z., H. Zhao, K. T. Peter, M. Gonzalez, J. Wetzel, C. Wu, X. Hu, J. Prat, E. Mudrock and R. Hettinger. 2021. A ubiquitous tire rubber–derived chemical induces acute mortality in coho salmon. Science. 371(6525): 185-189.